Reference: *Biol. Bull.* **165**: 221–240. (August, 1983)

ASCIDIAN-*PROCHLORON* SYMBIOSIS: THE ROLE OF LARVAL PHOTOADAPTATIONS IN MIDDAY LARVAL RELEASE AND SETTLEMENT

RICHARD RANDOLPH OLSON

Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

Abstract

Colonies of the algal-ascidian symbiosis *Didemnum molle* at Lizard Island, Australia, release more than 95% of their larvae daily between 11:00 and 14:00 with a peak around 12:30, shortly after meridian passage of the sun. In shallow-water habitats, larvae are photoadapted to lower light environments than are adult colonies. Unlike adult colonies, larvae lack spicules and brown pigmentation in their tunic. They also have a lower chlorophyll a/b ratio than do their parent colonies. In the field, larvae seek a light intensity of approximately 100 μE $m^{-2}s^{-1}$ and settle preferentially on dark or shaded substrata. Settled larvae that were transplanted into full sunlight perished after 4 days. Larvae observed in the field swam for less than 10 minutes before settling. When denied a shaded substrate, larvae swam for up to 1.5 hours and eventually settled in full sunlight (an unsuitable habitat). Larvae in total darkness swam for at least 2 hours before settling. The larval photoadaptations, settlement behavior, and mortality of *D. molle* juveniles in full sunlight suggest that the release of larvae at midday, when sunlight is greatest, enables larvae to search for settlement sites when conditions are most severe, minimizing the chance they will settle in unsuitable habitats.

Introduction

The availability of suitable habitats for the settlement of larvae of sessile marine invertebrates is known to vary spatially (Grosberg, 1981; Palmer and Strathmann, 1981; Sebens, 1981; Keough and Downes, 1982) as well as temporally (Grosberg, 1982). Although considerable research has been conducted on factors that induce larvae to settle (Meadows and Campbell, 1972), very little is known about the ecological significance of the time of day that larvae are released. Many species of sessile invertebrates have larvae that swim for less than an hour before settling [*e.g.*, some ascidians (Crisp and Ghobashy, 1971), bryozoans (Ryland, 1974), and corals (Lewis, 1974)]. Such a short time between larval release and settlement potentially enables the parent to control the time of day its larvae will settle.

Colonial ascidians are commonly members of fouling communities in temperate waters (Millar, 1971) and cryptic communities on coral reefs (Jackson, 1977). Eighteen species of one family (Didemnidae) possess symbiotic unicellular algae (Kott, 1980). These species are found only in the tropics and commonly occur, not in cryptic communities, but in fully sunlit areas on coral reefs. Although considerable research has been conducted on the symbiotic algae, there is little known about the ecology of the animals or their larvae.

Of the eighteen species of ascidian-algal associations, two species possess algae of the genus *Synechocystis* (Lafargue and Duclaux, 1979; Olson, 1980), a cyanophyte

Received 17 February 1983; accepted 25 May 1983.

which contains phycobilin pigments. The other species contain algae of the recently discovered genus *Prochloron* (Newcomb and Pugh, 1975). The algae are unique in that they have a cell structure (Whatley, 1977), cell wall (Moriarty, 1979), and genome which resemble procaryotes (Seewaldt and Stackebrandt, 1982), but contain chlorophyll b and lack phycobilin pigments, typical eucaryotic features (Lewin and Withers, 1975). This contradiction has led to their designation as a new genus and provisionary new division, the Prochlorophyta (Lewin, 1976).

Colonial ascidians studied to date, which do not have symbiotic algae, have been reported to release their larvae primarily at dawn or upon first light after a period of darkness. Of the thirteen species listed in Table I, nine release their larvae in the morning, two release at midday, and two release larvae throughout the day-night cycle. Duyl *et al.* (1981) (Table II) reported the first case of a species in which larval release takes place only during the midday hours. The larvae of this species, *Trididemnum solidum,* possess symbiotic algae and are released between 10:15 and 14:00. Here I report on another species of colonial ascidian with symbiotic algae which releases 95 percent of its larvae between 11:00 and 14:00. Experimental field

TABLE I

Larval release times for colonial ascidians without symbiotic algae

Species	Time of release	Location	Reference
Aplidium constellatum	dawn	Woods Hole, MA	Mast (1921)
	all morning	Woods Hole, MA	Scott (1924)
	dawn	Woods Hole, MA	Costello and Henley (1971)
Botrylloides mutabilis	morning	Tokyo Bay, Japan	Yamaguchi (1975)
Botrylloides nigrum	morning	Puerto Rico	Morgan (1977)
Botryllus schlosseri	all day with a peak at midday	Woods Hole, MA	Grave and Woodbridge (1924)
	all day with a peak at midday	Woods Hole, MA	Grave (1937)
Cystodytes lobatus	3–4 hours after dawn, all day	Pacific Grove, CA	Lambert (1979)
Diplosoma listerianum	all day, peak at midday	Menai Bridge, North Wales	Crisp and Ghobashy (1974)
Distaplia occidentalis	morning	Friday Harbor, WA	Watanabe and Lambert (1973)
Ecteinascidia turbinata	morning	Puerto Rico	Morgan (1977)
Leptoclinum mitsukurii	morning	Tokyo Bay, Japan	Yamaguchi (1975)
Metandrocarpa taylori	continuous over day/night cycle	Pacific Grove, CA	Abbott (1955)
	morning	Friday Harbor, WA	Watanabe and Lambert (1973)
Perophora viridis	early morning	Woods Hole, MA	Grave and McCosh (1924)
	8:00–11:00	Woods Hole, MA	Costello and Henley (1971)
Polyandrocarpa tincta	morning	Tortugas, FL	Grave (1936)
Symplegma viride	continuous over day/night cycle	Tortugas, FL	Grave (1937)

TABLE II

Larval release times of colonial ascidians with symbiotic algae

Species	Time of release	Location	Reference
Didemnum molle	11:00–14:00	Lizard Island, Australia	This paper
	midday	Palau, Caroline Islands	Olson, unpublished data
Diplosoma similis	midday	Lizard Island, Australia	Olson, unpublished data
Lissoclinum patella	midday	Palau, Caroline Islands	Lewin, pers. comm.
Lissoclinum voeltzkowi	11:45–13:30	Lizard Island, Australia	Olson, unpublished data
Tridemnum solidum	10:15–14:00	Curacao	Duyl *et al.* (1981)

evidence is presented showing that light intensity of the habitat in which a larva settles can be very important to its eventual growth and survival.

The colonial ascidian *Didemnum molle* Herdman, lives on coral reefs throughout the Indo-West Pacific (Kott, 1980). All colonies contain symbiotic algae of the genus *Prochloron,* which are extracellularly attached to the walls of the cloacal chambers of the ascidian. The algae are shielded from full sunlight by the ascidian tunic which contains spherical calcareous spicules (40–80 μm diameter) and a dark brown pigment (Fig. 1a). The larva of *D. molle* (Fig. 2a) is relatively large (2.5 mm length) and can be seen easily underwater. Its large size, midday release, short swimming time, and relatively large amount of algae (0.39 μg chlorophyll a/larva, s.d. = 0.09) enabled me to study aspects of its larval ecology in the field which have not been examined previously in an algal-invertebrate symbiosis.

MATERIALS AND METHODS

All experiments reported, unless otherwise noted, were conducted at a depth of 2 m on a patch reef approximately 200 m directly offshore of the Lizard Island Research Station, Lizard Island, Australia (14 40′ S. lat, 145 28′ N. long.) from August to December 1981. Few laboratory experiments were performed because *D. molle* colonies will seldom survive for more than a day in aquaria, and experiments conducted underwater on the reef more closely resemble the light and temperatures to which the larvae are acclimated. All times reported are local mean time (LMT) which is zone time corrected for longitude and the equation of time. LMT means that the sun is directly overhead at exactly 12:00.

According to Kott (1980), *D. molle* colonies may be brown or white in color. Recent findings (Olson, in prep.) suggest that the two color morphs are different species. Brown colonies and their larvae were used for all experiments reported in this paper.

"Settling panels" were 20 cm × 20 cm squares of 3 mm thick asbestos fiberboard with 3 cm long wooden legs at each corner. The legs supported the panels slightly above the substrate so that larvae could settle on the shaded undersides. "Juveniles" were sexually immature (less than 0.1 g wet weight) colonies that have a transparent tunic and are not heavily spiculated. Such colonies appear green due to the algae they contain (Figs. 2b, 2c). Sexual maturation occurs at 0.5 g wet weight (unpublished data). "Edge distance" is the distance between a newly settled larva on the underside of a settling panel and the nearest edge of the panel.

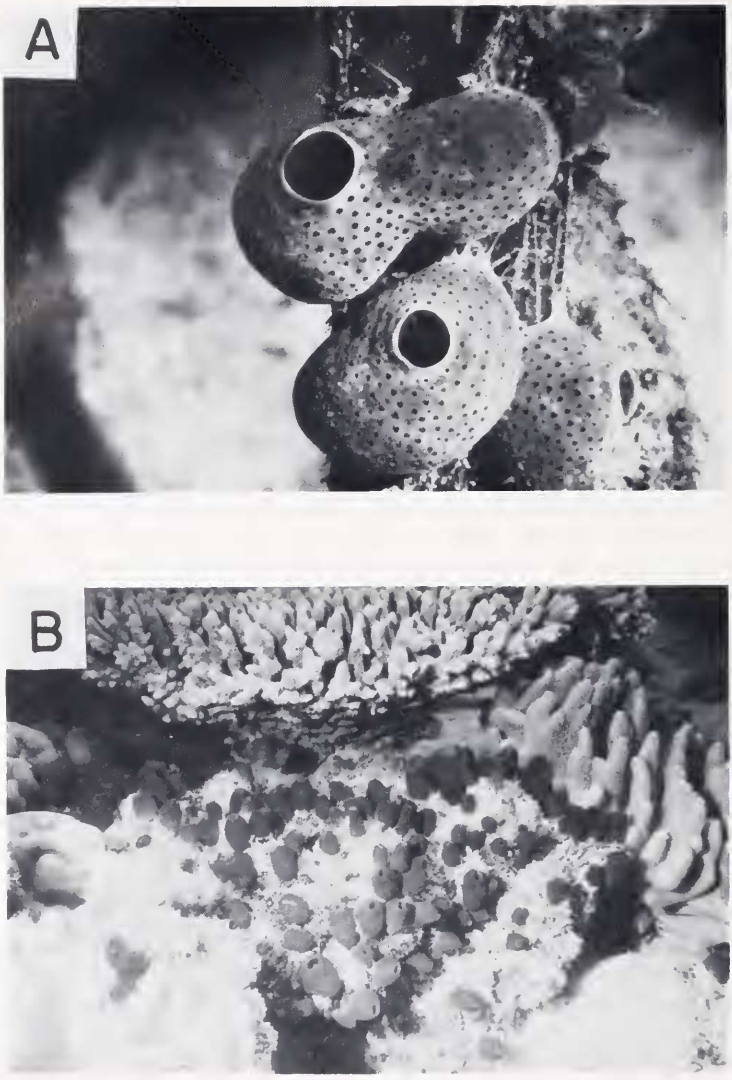

FIGURE 1. A—Adult colonies of *D. molle* fully expanded showing their single large exhalent opening and many, small inhalent openings of individual zooids. Note hair-like projections from edge of lower colony. These are extensions of the test, used for whole colony movement. B—*D. molle* habitat. This aggregation of approximately 150 colonies at 2 m depth was used for larval release observations. Colonies are in full sunlight. Juvenile colonies could be found on the underside of the boulder to which the colonies are attached.

Field observations of larval release

The timing of larval release was studied by observing one clump of approximately 150 colonies, closely aggregated on a piece of coral rubble approximately 0.5 m $\times$ 0.5 m across. Colonies were observed continuously from 11:00 to 15:00 for three consecutive days every two weeks (on the full and new moons) from August to December, 1981. As larvae were released from the colonies their time of release was recorded. The data were grouped into 15 minute intervals.

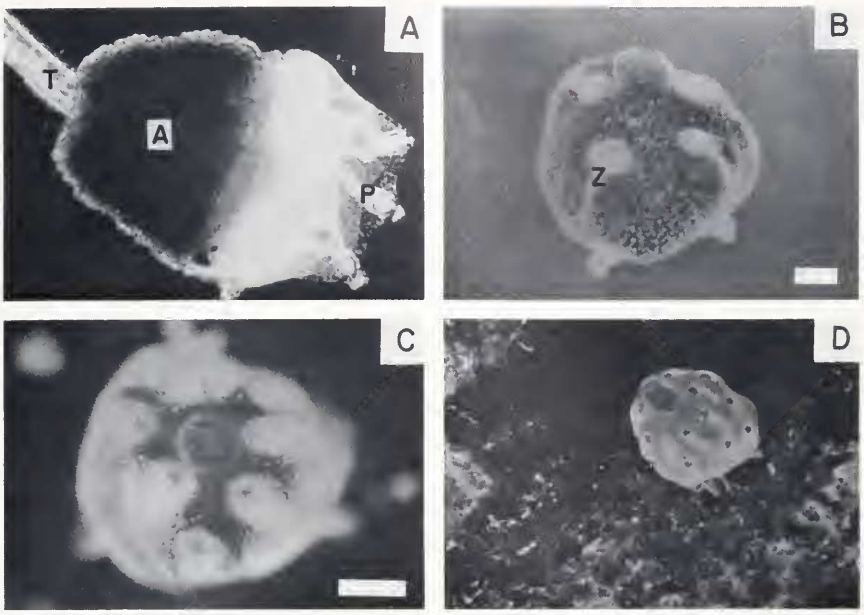

FIGURE 2. A—Larva of *D. molle*. Note three adhesive papillae (P). *Prochloron* algae (A) are attached to hair-like projections from the posterior end of the larval body according to Kott (1980). Distance from the base of the tail (T) to the tip of the middle adhesive papilla is approximately 1 mm. Larva contains three blastozooids. B—3 day-old juvenile. The colony has three zooids. White areas are spicules. Note that spicules are located around zooids (Z). The rest of the colony is green from the algae. Individual algal cells can be seen in this photo. Protrusions of test at base of colony are used for whole colony locomotion. Bar equals 0.25 mm. C—12 day-old juvenile. The colony has approximately 9 zooids. This photo was taken in the laboratory so colony is tightly contracted. The colony has no brown pigmentation. Bar equals 0.5 mm. D—Field photo of colony of approximately 30 zooids fully expanded. This colony was found on the underside of a coral rubble boulder at 2 m depth. In the original color photo, the colony can be seen to have a small amount of brown pigmentation on its topside.

Chlorophyll determinations

Chlorophyll content of whole colonies was measured by macerating individual colonies in the dark in 20 to 40 ml (depending on colony size) of 90 percent acetone buffered with $MgCO_3$. Samples were extracted overnight at 10°C, then centrifuged for 5 minutes at 2200 g. Samples were analyzed on a Varian spectrophotometer for absorbance at 647 and 664 nm wavelengths. Chlorophyll a and b concentrations were calculated with the equations of Jeffrey and Humphrey (1975).

Chlorophyll content of larvae was measured by the same methods. Larvae were collected as they were released from freshly collected colonies in aquaria. 5 ml of 90 percent acetone were used for extraction of groups of 30 to 40 larvae.

Light intensity measurements

Light intensities were measured using a Li-Cor light meter with a cosine corrected submersible quantum sensor. All measurements were taken on a calm, cloudless day in February between 12:00 and 13:00. To estimate light levels beneath the settling plates, holes were drilled at different distances from the edge. The holes were the same diameter as the light probe so that the plate could be placed on the bottom

with the light probe inserted upside down. The holes were drilled at 8, 20, and 35 mm distances from the edge. The first two distances correspond to the mean distances of larval settlement at 4 and 2 m depths, respectively. Light readings were taken at 2 and 4 m depths.

Survivorship experiment

The importance of settling in a shaded habitat was investigated by comparing survivorship of juveniles placed in a variety of light conditions. Larvae were allowed to settle on the undersides of settling panels at 2 m depth. The panels were inverted and subjected to one of the following three treatments: 1) shade—panel was covered by another panel of identical dimensions mounted 3 cm above it, 2) full sunlight, 3) clear plexiglas roof—a control for alterations in sedimentation and flow in the shade treatment. Juveniles on the undersides of uninverted panels served as controls for the inversion. Survivorship was recorded after four days. The experiment was replicated three times.

Swimming experiments

To determine how long larvae are capable of swimming, two experiments were conducted—one in the lab, the other in the field. In the field experiment, larvae were captured underwater with a 10 ml plastic syringe just as they were released from their parent colony. Each replicate consisted of ten larvae captured within 2 minutes time to assure that they were all at approximately the same developmental stage. The larvae were injected into plastic boxes measuring 12 cm $\times$ 8 cm $\times$ 6 cm. In the dark treatment, the entire box was painted black on the outside with latex paint and wrapped with two layers of aluminum foil. The dark roof treatment was a clear box with the outside of the roof of the box painted black. The shade treatment was a clear box placed 20 cm beneath a 80 cm $\times$ 60 cm white plastic shade. The clear treatment was a clear box left in full sunlight. Each treatment was checked every ten minutes for one hour except for the dark treatment which was checked only at the end.

In the lab experiment, larvae were obtained from freshly collected colonies in aquaria and placed into 500 ml beakers which were then wrapped in black plastic and placed in a darkroom. After two hours the beakers were uncovered and the larvae were censused.

Larval swimming observations

Larval swimming times were recorded for 89 larvae by visually tracking the larvae underwater using scuba gear. Although eighty-four percent of the larvae followed were lost as they swam among corals or when they reached the surface water where surface waves would toss them around, 14 larvae were followed all the way to settlement. The longest that any larvae were followed was 15 minutes, with the exception of one larva which was swept off of the reef and swam for over 25 minutes over the sand flats.

Results

Larval release observations

The larvae of *D. molle* are easily visible underwater. They are approximately 2.5 mm total length with the main body approximately 1 mm in length (Fig. 2a).

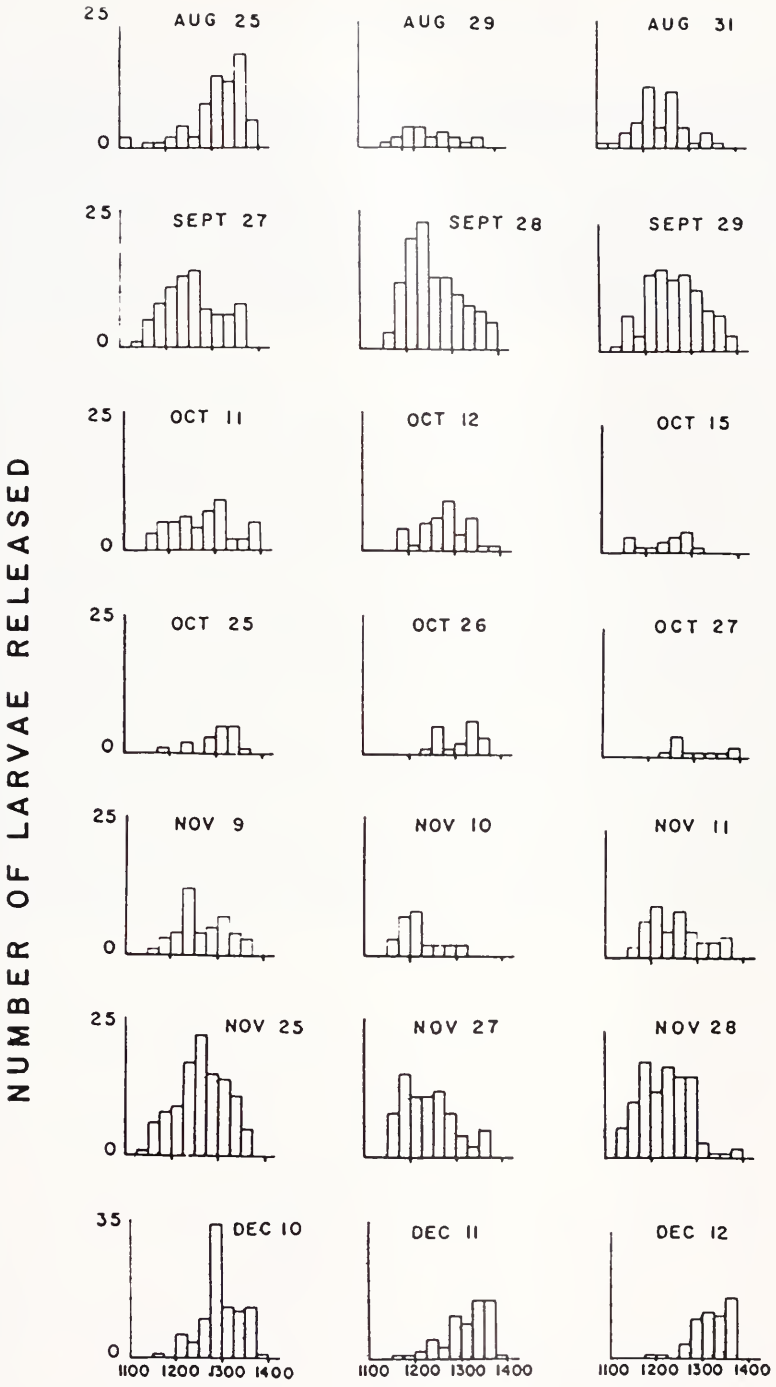

FIGURE 3. Daily larval release observations of 1981 showing the consistency of midday release times between days. The ordinate is number of larvae released during each 15 minute interval from the group of approximately 150 colonies shown in Figure 2B. The total number of colonies observed varied from day to day due to colonies dividing, migrating, and mortality.

The clump of bright green *Prochloron* algae attached to the larva make it easily discernable as a dark spot against a light background. Larvae release themselves by rupturing through the wall of the common cloaca where they have been developing, then swim vigorously out of the large common cloacal aperture (Kott, 1980). Similar to other colonial ascidian larvae (Millar, 1971), *D. molle* larvae are attracted to bright light during the beginning of their swimming stage. Larvae generally swim towards the surface, then drift back downward. After a short period of time (1–10 minutes) the larvae begin to seek dark surfaces. When visually following larvae, I had to maintain a distance of at least 0.5 m to prevent them from swimming towards my black wet suit.

A total of 88 larvae were followed in the field from their time of release. Of these, 14 were followed all the way to settlement. Their swimming times ranged from 40 seconds to 370 seconds, with a mean of 201 seconds (s.d. = 121). This value is, of course, skewed to the lower end since longer swimming larvae have a lesser chance of being followed all the way to settlement. However, it does show that many larvae swim for a very short period of time.

Of the 14 larvae that were followed all the way to settlement, twelve settled on the undersides of coral rubble and two settled on polyps of *Porites* coral. Fourteen observations were made of pomacentrid fish ingesting *D. molle* larvae. In all instances, the larvae were immediately egested and continued to swim, apparently unharmed. One larva disappeared into the inhalent siphon of a solitary ascidian (*Polycarpa* sp.). Several larvae were observed temporarily snagged on coral tentacles (acroporids and poritids), but they managed to free themselves. It thus appears that there are no major predators on the swimming stage of *D. molle*.

Larvae are released near midday. During a two week period larval settlement on settling plates was monitored every day at 11:00 and 16:00. Ninety-three percent of the recruitment took place during the midday interval. During hundreds of hours underwater, no larvae were ever seen before 10:30 or after 15:00. Figure 3 shows clear peaks in the daily time of larval release. The compilation of this data (Fig. 4)

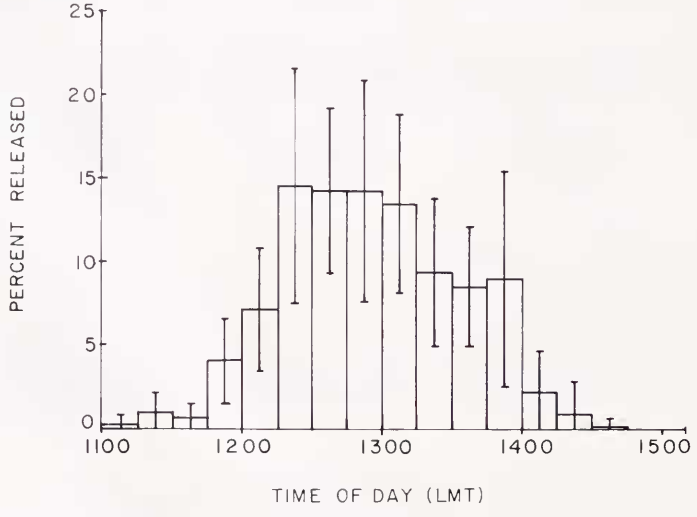

FIGURE 4. Compiled larval release times for all days of observation in which more than 40 larvae were released (N = 15 days, 1126 larvae). Bars represent 95% confidence limits. Note that 95% of all larvae were released between 11:00 and 14:00. Mean time of larval release is 12:54 (S.E. = 12.7 minutes).

gives a mean time of release of 12:54 (SE = 12.5 minutes). The data do not differ significantly from a normal distribution [Kolmogorov-Smirnoff, dmax = 0.053, $P > 0.1$ (Sokal and Rohlf, 1969)].

The symbiotic algae of *D. molle* are extracellular to the animal host. In an adult colony, the algae line the walls of the common cloaca. In a larva, the algae are attached to small hairlike projections at the posterior end of the larval body (Kott, 1980). Although no data exist on the physiological importance of the algae to the larva, it is doubtful that they contribute much to the larva's nutrition, considering their external location.

Photoadaptations of the algae

The ratio of chlorophyll a to chlorophyll b is generally regarded as a relative indicator of the light levels to which a plant is photoadapted (Boardman, 1977). Chlorophyll b is an accessory photosynthetic pigment, absorbing light primarily around 470 nm and 650 nm. It is usually produced in higher quantities in lower light environments. This appears to hold true for the *D. molle* colonies analyzed (Fig. 5). Adult colonies living deeper have greater amounts of chlorophyll b relative to chlorophyll a. However, examination of larvae collected from shallow water colonies (2 m depth) shows that their chl a/chl b ratio is less than that of their parent colonies. The larvae thus appear to be photoadapted to lower light regimes than the habitat of the parent colonies.

How can the larval algae have a lower chl a/chl b ratio than the parent from which it was released? Although current research suggests that some phytoplankton can alter their chlorophyll ratio in very short periods of time (Falkowski, 1980), it is doubtful that this is the case for *D. molle* larvae since they have such a brief swimming period and are exposed to a wide range of light intensities. The values presented in Figure 5 represent averages for extracts from whole colonies. There is undoubtedly a great deal of self-shading within a colony so that much less light

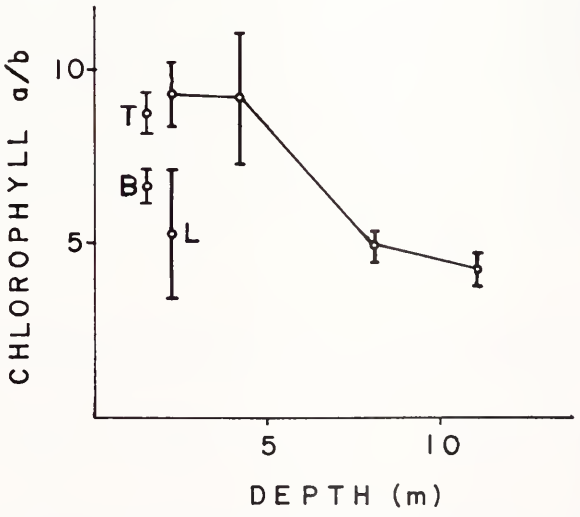

FIGURE 5. Chlorophyll a/b ratios of adult colonies (unlabeled points, N = 5 for each point), larvae (L) (N = 8 extractions of 30–40 larvae), and 3 colonies bisected into top (T) and bottom (B) halves. Error bars are standard deviations.

reaches the bottom of the colony than the top. Three colonies from 2 m depth were bisected with a razor blade into upper and lower halves. Each portion was analyzed for chlorophylls. The results are points T (top) and B (bottom) in Figure 5. The lower half has a chl a/chl b ratio approaching the larvae, suggesting that the larvae gather their algae from the lower portion of the colony. Unfortunately, it was not possible to observe larvae within the colony previous to release.

Substrate choice experiments

In the substrate choice experiment (Table III), the larvae almost unanimously chose the dark substrata, indicating that they are capable of differentiation between dark and light surfaces.

Larvae settled in a somewhat distinct band around the outer edge of the undersides of settling panels. Figure 6 shows the frequency distributions of larvae settled on panels at 2 and 4 m depths. Table IV gives the mean values for the edge distances. Although there was considerable variation, there was a clear tendency for larvae to settle closer to the edge at the deeper site. Comparison of observed edge distances with the expected distribution based on random settlement and area alone, shows that larvae settled predominantly near the edge (Fig. 7).

Light intensities were measured beneath the settling plates at the mean edge distances (Table IV) of settled larvae. The light intensity at the mean edge distance measured at the shallow and deep sites was 100 and 110 μE $m^{-2}s^{-1}$, respectively (Fig. 8). Thus larvae appear to seek a light intensity of approximately 100 μE $m^{-2}s^{-1}$. This means that at deeper sites, where light intensity on the top of surfaces is less than 100 μE $m^{-2}s^{-1}$, larvae should settle on the upper surfaces of substrata. At Lizard Island, this light intensity occurs around 15 m depth. At this depth, juveniles were found living in unshaded sites.

There are rare instances when newly settled larvae are found in unshaded habitats in shallow water, but these are certainly the exception. As a part of another study of *D. molle* recruitment at Lizard Island, settlement of larvae on settling panels at 2 m depth was recorded over five days every two weeks. Of over 3000 settled larvae, only three settled on the topside of the settling panels. The rest settled on the undersides or occasionally on the legs of the panels. Juvenile colonies were never observed living in full sunlight. They are generally found on the undersides of coral plates. Figure 9 shows the size distribution of *D. molle* colonies on the topside and underside of a coral plate collected from 2 m depth. The 0.1 gm size class of the underside population was composed primarily of newly settled juveniles, still green in color. None of the topside colonies were green.

TABLE III

*Substrate choice experiment**

		Roof				
Trial	N	Black	White	Bottom	Side	Not Attached
1	35	65	0	6	3	26
2	33	40	3	30	24	3
3	71	79	0	15	6	0

* Roof of chamber was painted with black and white squares. Data are percent of all larvae in treatment (N).

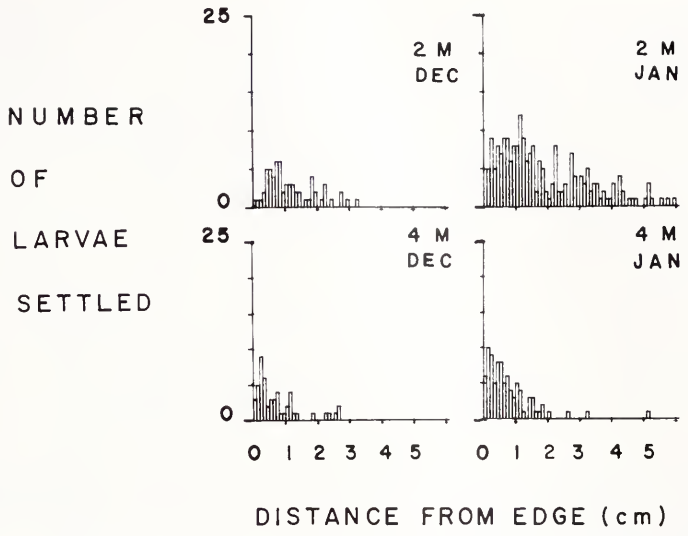

FIGURE 6. Distributions of edge distances of larvae that settled on the undersides of settling panels at 2 and 4 m depth during one week in December, 1981, and January, 1982.

Survivorship experiment

Four days of full sunlight was lethal to the newly settled larvae (Table V). Healthy juveniles are colored bright green by their symbiotic algae. They have few spicules and thus the algae can be seen through the tunic. Juveniles exposed to full sunlight changed from bright green to light green to grayish-brown, then withered and died. Colonies in the shade appeared healthy and of normal size and color, as did the control colonies. Colonies beneath the clear plexiglas roof did not die as rapidly as those fully exposed, probably because they were shaded by a small amount of sediment which accumulated each day on the plexiglas roof. They did, however, show the same evidence of deterioration and their mortality was also high. This is interesting since plexiglas is an effective filter of ultraviolet radiation (Jagger, 1977).

Larval swimming time experiments

D. molle larvae are capable of swimming for more than an hour if they do not find a suitable site for settlement (Fig. 10). In the clear treatment, all nonsettled

TABLE IV

*Mean edge distances ($\bar{X}$) of larvae settled on the bottoms of settling panels at 2 and 4 m depths**

Depth (m)		December sample	January sample
2	$\bar{X}$	1.75	1.95
	s.d.	1.09	1.55
4	$\bar{X}$	0.80	0.78
	s.d.	0.70	0.78

* Measurements are cm from the edge of the panel. s.d. = standard deviation.

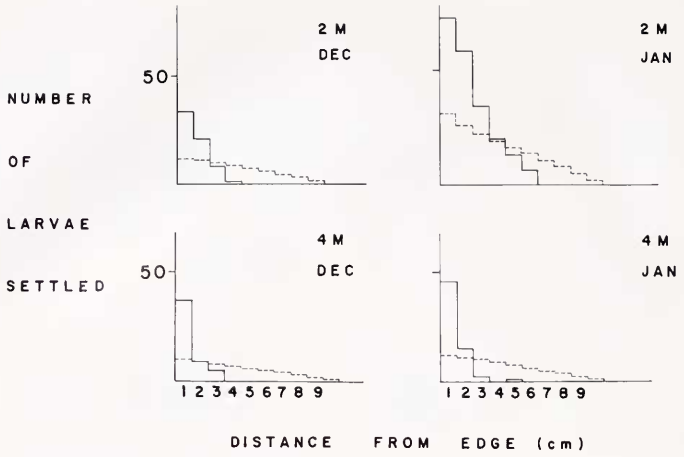

FIGURE 7. Comparison of observed (solid line) and expected random (dashed line) distributions of edge distances of settled larvae. Expected distributions were calculated by multiplying the total number of larvae by the amount of area in the distance interval.

larvae were lying on the bottom of the chamber after one hour, only occasionally swimming up off the bottom. By 1.5 hours, all larvae had ceased swimming. This is in contrast to the dark experiment in the lab (Table VI) in which almost half of the larvae were still active after two hours. Thus it appears that larvae can swim longer in lower light environments. This would be important to larvae swept into deeper water.

What happens if larvae do not find a suitable substratum for settlement? It has already been shown that larvae prefer dark substrata over light (Table III). This result is seen again in the larval swimming experiment (Fig. 10). In the roof and shade treatments the majority of larvae settled within the first twenty minutes. The

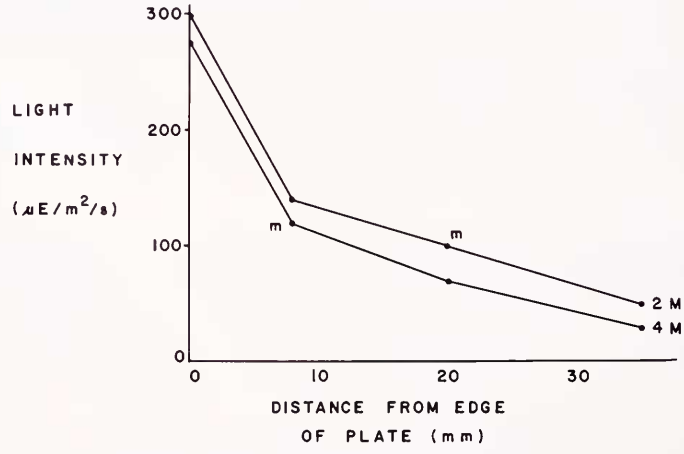

FIGURE 8. Light intensities measured on the undersides of settling panels. Points labeled "m" correspond to mean "edge distances" of settled larvae at respective depths.

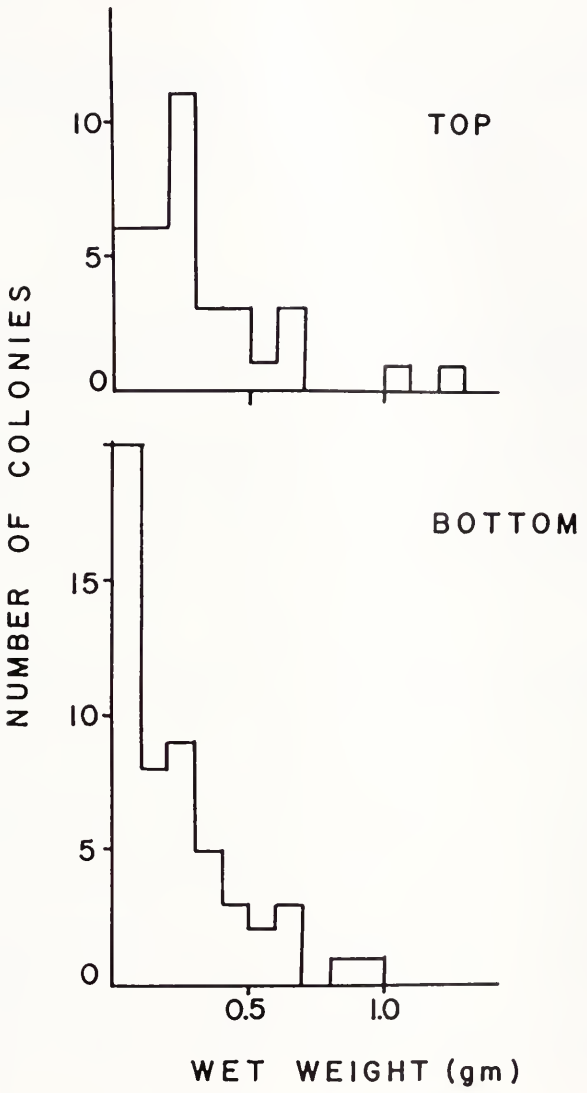

FIGURE 9. Size distributions of colonies on the top and bottom of a dead coral plate collected from 2 m depth. Note that the largest size class of colonies on the underside of the plate is the 0.1 g size group.

larvae settled primarily on the top of the chamber in the roof and shade treatments (Table VII).

In the clear treatment, where larvae were given no dark substrata or shade, the larvae swam continuously upward. After about 45 minutes, most of the larvae lay on the bottom, still swimming, but seldom raising above the bottom. Eventually most of these larvae attached themselves to the bottom where they metamorphosed. The survivorship experiment (Table V) showed that an unshaded settlement site is lethal. Thus the denial of a suitable site (shade) eventually results in the larvae settling in a much less suitable or certain-death habitat.

TABLE V

*Juvenile survivorship experiment**

Treatment	Total number of larvae	Mean % survivorship after 4 days	Standard deviation	Level of significance
Shade	34	77.3	28.0	—
Control	54	82.2	12.6	N.S.
Clear plexiglas	35	38.3	20.5	$P < 0.01$
Full sunlight	28	2.7	4.6	$P < 0.001$

* See text for explanation of treatments. Data tested for significant difference from shade treatment using single factor analysis of variance ($F_s = 12.23$, $P < 0.01$) with Student-Newman-Keuls multiple comparisons test. Data were arcsine transformed (Zar, 1974). Each treatment was replicated 3 times.

The ability of larvae to delay their settlement is important in an habitat like the Lizard Island lagoon. Patch reefs provide plenty of suitable habitats for the larvae, but between them lie bright white sand flats with little or no shaded substrata. By postponing settlement, larvae can drift over the sand flats until they encounter another patch reef, thus achieving inter-reef recruitment.

DISCUSSION

The larval stage of *Didemnum molle* is not substantially different from the typical colonial ascidian larval phase as described by Millar (1971). Upon release, larvae are positively phototactic, swimming towards bright light. They gradually change to negative phototaxis and negative geotaxis, swimming upwards and settling on the undersides of dark surfaces. What appears to be distinguishing about *D. molle* (and perhaps all ascidian-algal associations, see Table II) is that the larvae are released only in the middle of the day with a peak shortly after meridian passage of the sun. This phenomenon held true for *D. molle* during the three seasons (spring, summer, winter) in which it was studied. Duyl *et al.* (1981) reported a similar midday timing of larval release for *Trididemnum solidum*, a Caribbean ascidian-algal symbiosis.

Larval release by colonial ascidians has previously been reported to occur primarily at dawn or first light after a long period of darkness (Table I). However, as Kott (pers. comm.) notes, most of the ascidians studied have been temperate species. In all of the papers cited in Table I, there is little speculation as to the functional

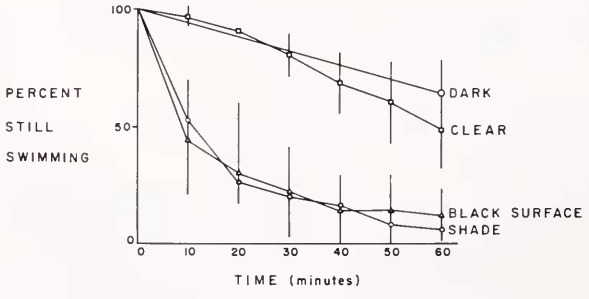

FIGURE 10. Larval swimming time experiment. See text for explanation of treatments. Bars represent 95% confidence limits. Dark treatment was examined only at 60 minutes. Each treatment was replicated four times, except the dark treatment (N = 3). Each replicate contained ten larvae.

TABLE VI

Percent larvae swimming (sw), metamorphosed and attached (m/a), and metamorphosed but not attached (m/na) after two hours in total darkness

Trial	N	sw	m/a	m/na
1	25	48	40	12
2	16	44	44	12
3	12	33	33	33

significance of the timing of larval release. Watanabe and Lambert (1973) noted that the larvae of *Distaplia occidentalis* are released only during daylight and primarily in the morning. Their behavior and settlement is closely attuned to light conditions, with the larvae settling in dark habitats. This presumably enables them to find cracks and crevices which provide refuge from predators and physical stress such as strong currents. But no experiments were performed to test whether survivorship is greater in cracks and crevices.

Experiments with the larvae of *D. molle* suggest a clear purpose for the midday timing of larval release. The light intensity of the juvenile habitat appears to be a very important (if not the most important) factor determining the suitability of the settlement site. Too much light is lethal to the juvenile (Table V), too little light reduces the growth and photosynthetic rate of the algae which probably has a direct effect on the growth of the ascidian. By releasing larvae at midday, when light intensity is greatest, adult colonies enable their larvae to search for settlement sites

TABLE VII

*Settlement sites of larvae in swimming experiment conducted on reef**

		Larval settlement site									
Treatment		Sw	T	B	Si	M	Multiple comparison				
Clear	X%	47.5	10.0	30.0	12.5	0.0	Sw	B	Si	T	M
	$X\%_a$	47.5	5.0	24.0	12.23	0.0					
	s_a	1.0	7.7	11.8	0.5	0.0					
Shade	X%	5.0	45.0	15.0	35.0	0.0	T	Si	B	Sw	M
	$X\%_a$	2.6	43.6	2.0	28.8	0.0					
	s_a	3.4	14.2	11.4	19.1	0.0					
Black Surface	X%	12.5	50.0	27.5	7.5	2.5	T	B	Sw	Si	M
	$X\%_a$	9.1	49.6	25.4	3.8	0.6					
	s_a	5.5	5.1	5.7	5.4	2.5					
Dark	X%	3.3	6.7	20.0	56.7	13.3	Sw	B	M	T	Si
	$X\%_a$	0.3	1.4	11.7	56.8	5.3					
	s_a	1.0	4.0	8.8	1.4	4.0					

* See text for explanation of treatments. Results of treatments underlined at right were not significantly different from each other using Student-Newman-Keuls multiple comparisons test ($P < 0.05$). N = 4 trials for each treatment, except for dark treatment (N = 3). Each trial included ten larvae. Sw—larvae still swimming; T—settlement on top of chamber; B—settlement on bottom of chamber; Si—settlement on side of chamber; M—larvae that metamorphosed but did not attach; X%—mean percent settlement; $X\%_a$—mean percent settlement using arcsin transform on data, then back transforming; s_a—standard deviation of transformed data.

under the most extreme conditions, minimizing the chance of settling in a location that is too bright.

Given that the algae within the parent colony are photoadapted to different light levels according to their depth in the colony (Fig. 5), why should the larvae have evolved to collect algae from the more shade adapted portion of the parent colony? The larvae and juveniles lack the photoadaptations of the adult colonies and thus must settle in a low light habitat. Adult colonies contain calcareous stellate spicules [40–80 μm in diameter (Kott, 1980)] and a dark brown animal pigment (D. Parry, pers. comm.) in the outer test of the colony. These materials shield the algae from much visible and ultraviolet radiation. The test of the larvae and young juveniles is transparent, lacking both the spicules and brown pigment. Juveniles are the color of their symbiotic algae due to this transparency. After two days, juveniles begin to produce spicules which originate around each zooid (Fig. 2b). Although calcareous spicules are found in many colonial ascidians without symbiotic algae (Van Name, 1945), in the algal-ascidian symbioses they appear to have been modified into a photobiological role. The Caribbean species *Trididemnum solidum* produces a significantly higher proportion of spicule versus tissue in higher light intensity habitats (Olson, 1980).

At about two weeks of age, brown pigmentation begins to appear in the test of juvenile *D. molle* colonies. Experiments by Jokiel (1980) have demonstrated a lethal effect of ultraviolet radiation on invertebrates that normally live in the shade. *D. molle* has obviously evolved a means of protecting itself in the adult stage from the damaging effects of ultraviolet radiation. It is probably the spicules and brown pigment that achieve this. By gathering shade-adapted algae prior to release from the parent colony, the larva is prepared to settle in a low light environment and thus does not require spicules and brown pigment for a shield. The small size of the larvae probably prohibits them from already possessing these photoadaptations upon release.

Examination of dead coral plates and rubble on the shallow reefs around Lizard Island shows that the undersides are the nurseries for *D. molle* (Fig. 9). Juvenile colonies (green in color) are found only on the undersides of such substrata. It appears that juveniles grow on the undersides until they have acquired the proper photoadaptations (spicules and pigment), then migrate around the edge of the coral plates into the full intensity of sunlight (up to 2600 μE $m^{-2}s^{-1}$) (Fig. 11). Colonial ascidians have long been known to move, through the extension of stolonic test vesicles (Carlisle, 1961). Birkeland *et al.* (1981) documented whole colony movement by didemnid ascidians in general, and Cowan (1981) reported movement by *D. molle*. I attempted to follow juveniles through this stage in the field by placing markers beside them, but this proved impossible since it required daily monitoring for more than a month. When occasional rough weather interrupted the monitoring, the colonies were lost. Nevertheless, juveniles are found on the undersides and adults are found mostly on the topsides (Fig. 9). The migration probably takes several weeks.

Larval ecology of algal-invertebrate symbioses

Relatively little research has been conducted on the larval ecology of algal-invertebrate symbioses. The findings of this study of *D. molle* are relevant to other invertebrates with lecithotrophic larvae containing algae. These include corals such as *Pocillopora damicornis* (Edmundson, 1929; Kawaguti, 1941; Harrigan, 1972), *Seriotopora hystrix* (Atoda, 1951), and *Favia fragum* (Lewis, 1974).

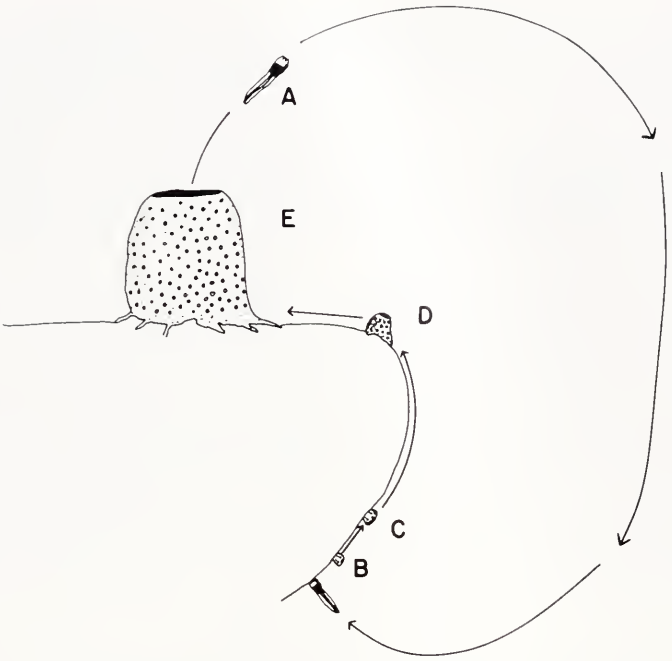

FIGURE 11. The life cycle of *D. molle*. Stages A–D correspond to photos in Figure 1. Larvae are released and settle at midday. Tail resorption by the larva is completed approximately 20 minutes after attachment. Complete metamorphosis takes approximately 3 hours. By late afternoon most colonies have three functioning zooids (B). At six days all zooids divide to form a total of 6 zooids. Another synchronous division usually occurs at 12 days, after which division is asynchronous. As a colony produces more spicules and acquires brown pigmentation (D) it presumably migrates into full sunlight (E).

Lewis (1974) studied settlement of the hermatypic coral *Favia fragum* in the laboratory. The larvae showed a preference for dark surfaces and settled primarily on the undersides of substrata in dishes. When the substrata were inverted shortly after settlement, the larvae detached themselves and moved to the underside again. Lewis, surprised that a photosynthetic organism would settle in the shade, conjectured that it was probably a predator avoidance phenomenon. No consideration was given to the differences in photoadaptations between the adults and larvae. It is possible that the juveniles of *F. fragum,* similar to *D. molle,* cannot survive in full sunlight. Harrigan (1972) found that the larvae of *P. damicornis* also prefer to settle on dark surfaces.

Goreau *et al.* (1981) examined settling patterns and mortality of planulae larvae from the coral *Porites porites.* Larvae which settled on the sides of aquaria had a much higher mortality rate than those which settled on the bottom. They suggested that this might be due to reduced food availability. However, no consideration was given to light as a factor. Juveniles on the side were illuminated from all sides, whereas those on the bottom were dark on their undersides as well as being deeper in the water. Survivorship of larvae in shade was not investigated. Mortality was greatly reduced once the juveniles produced a skeleton. This may be analogous to spicule production in *D. molle* juveniles which results in a tolerance for greater light intensities.

Birkeland (1977) found that Caribbean corals at 9 m depth recruited more frequently on the sides and undersides of cement blocks than upper surfaces. At deeper sites the recruitment shifted towards the upper surface. Birkeland *et al.* (1981) report the same result for Pacific corals around Guam. His explanation is that macroalgae and sediment on the upper surface inhibit coral larval settlement. No mention is made of possible differences in photoadaptations between larvae and adults. The shift towards the upper surfaces at greater depths is similar to larval settlement edge distances for *D. molle* (Figs. 6, 7). Loya (1976) also found that the coral *Stylophora pistilata* settled and survived primarily on the undersides of surfaces in shallow-water habitats at Eilat, Israel.

Competition for space among sessile invertebrates is a popular explanation of community structure on coral reefs (Lang, 1973; Jackson and Buss, 1975; Connell, 1976; Bak *et al.*, 1977; Sheppard, 1979; Benahayu and Loya, 1981). It has been assumed that adult sessile invertebrates usurp potential larval settlement area (Maguire and Porter, 1977; Benahayu and Loya, 1981). If coral planulae are unable to withstand full sunlight, then adults may generate more suitable settlement space (their shade and undersides) than they consume. For the colonial ascidian *Didemnum molle,* there is little overlap between the habitats of the adults and newly metamorphosed larvae. Many other algal-invertebrate symbioses, upon close inspection, may follow the same pattern.

Acknowledgments

The Lizard Island Research Station and the Australian Institute of Marine Sciences generously provided research facilities. C. Cavanaugh, C. Crockroft, P. Filmer-Sankey, B. and L. Goldman, V. Harriot, M. Harriss, T. Givnish, R. Grosberg, R. Pardy, M. Patterson, H. Sweatman, K. Sebens, and L. Vail contributed helpful discussion. Figure 2a was kindly provided by P. Parks. P. Mather donated collecting materials. M. Harriss, H. Olson, and M. Patterson gave endless moral support for which I am grateful. Lastly, I thank R. T. Paine and T. Suchanek for their initial encouragement. This research was supported by the Franklin B. Knox Fellowship of Harvard University and NSF grant OCE 81-14299.

LITERATURE CITED

Abbott, D. P. 1955. Larval structure and activity in the ascidian *Metandrocarpa taylori. J. Morphol.* **97:** 569–594.

Atoda, K. 1951. The larval and postlarval development of some reef building corals. V. *Seriotopora hystrix* Dana. *Sci. Rep. Tohoku Univ. Fourth Ser.* **19:** 33–39.

Bak, R. P. M., J. J. W. M. Brouns, and F. M. L. Hays. 1977. Regeneration and aspects of spatial competition in the scleractinian corals *Agaricia agaricites* and *Montastrea annularis. Proc. 3rd Int. Symp. Coral Reefs* **1:** 143–148.

Benayahu, Y., and Y. Loya. 1981. Competition for space among coral-reef sessile organisms at Eilat, Red Sea. *Bull. Mar. Sci.* **31**(3): 514–522.

Birkeland, C. 1977. The importance of rate of biomass accumulation in early successional stages of benthic communities to the survival of coral recruits. *Proc. 3rd Int. Symp. Coral Reefs* **1:** 15–21.

Birkeland, C., L. Cheng, and R. Lewin. 1981. Motility of didemnid ascidian colonies. *Bull. Mar. Sci.* **31:** 170–173.

Birkeland, C., D. Rowley, and R. H. Randall. 1981. Coral recruitment patterns at Guam. *Proc. 4th Int. Coral Reef Symp.* **2:** 339–344.

Boardman, R. K. 1977. Comparative photosynthesis of sun and shade plants. *Ann. Rev. Plant Physiol.* **28:** 355.

Carlisle, D. B. 1961. Locomotory powers of adult ascidians. *Proc. Zool. Soc. Lond.* **136:** 142–146.

CONNELL, J. H. 1976. Competitive interactions and the species diversity of corals. Pp. 51–58 in *Coelenterate Ecology and Behavior,* G. O. Mackie, ed. Plenum Press, N. Y.

COSTELLO, D. P., AND C. HENLEY. 1971. *Methods for obtaining and handling marine eggs and embryos,* 2nd ed. Mar. Biol. Lab., Woods Hole, MA.

COWAN, M. E. 1981. Field observations of colony movement and division of the ascidian *Didemnum molle. Mar. Ecol. Prog. Ser.* **6:** 335–337.

CRISP, D. J., AND A. F. A. A. GHOBASHY. 1971. Responses of the larvae of *Diplosoma listerianum* to light and gravity. Pp. 443–465 in *4th European Mar. Biol. Symp.,* D. J. Crisp, ed.

DUYL, F. C. VAN, R. P. M. BAK, AND J. SYSBESMA. 1981. The ecology of the tropical compound ascidian *Trididemnum solidum.* I. Reproductive strategy and larval behaviour. *Mar. Ecol. Prog. Ser.* **6:** 35–42.

EDMONDSON, C. H. 1929. Growth of Hawaiian corals. *Bull. Bernice P. Bishop Mus.* **58:** 1–38.

FALKOWSKI, P. G. 1980. Light-shade adaptation in marine phytoplankton. Pp. 531–549 in *Primary Production in the Sea,* P. G. Falkowski, ed. Plenum Press, New York.

GOREAU, N. I., T. J. GOREAU, AND R. L. HAYES. 1981. Settling, survivorship and spatial aggregation in planulae and juveniles of the coral *Porites porites* (Pallas). *Bull. Mar. Sci.* **31**(2): 424–435.

GRAVE, C. A. 1936. Metamorphosis of ascidian larvae. *Carnegie Inst. Wash. Pap. Tortugas Lab.* **29:** 209–291.

GRAVE, C. A. 1937. Notes on the culture of eight species of ascidians. Pp. 560–564 in *Culture Methods for Invertebrate Animals,* P. S. Galtsoff, F. E. Lutz, P. S Welch, and J. G. Needham, eds. Comstock Press, Ithaca, NY.

GRAVE, C. A., AND G. MCCOSH. 1923. *Perophoris viridis* Verrill: The activities and structure of the free swimming larvae. *Wash. Univ. Stud. Sci. Ser.* **11:** 89–116.

GRAVE, C. A., AND H. WOODBRIDGE. 1924. *Botryllus schlosseri* (Pallas): The behavior and morphology of the free swimming larva. *J. Morphol.* **39:** 207–247.

GROSBERG, R. K. 1981. Competitive ability influences habitat choice in marine invertebrates. *Nature* **290:** 700–702.

GROSBERG, R. K. 1982. Intertidal zonation of barnacles: the influence of planktonic zonation of larvae on vertical distribution of adults. *Ecology* **63**(4): 894–899.

HARRIGAN, J. F. 1972. Behavior of the planula larva of the scleractinian coral *Pocillopora damicornis* L. *Am. Zool.* **12:** 723.

JACKSON, J. B. C. 1977. Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. *Am. Nat.* **111:** 743–767.

JACKSON, J. B. C., AND L. W. BUSS. 1975. Allelopathy and spatial competition among coral reef invertebrates. *Proc. Nat. Acad. Sci. U.S.A.* **72**(12): 5160–5163.

JAGGER, J. 1977. Phototechnology and biological experimentation. Pp. 1–26 in *The Science of Photobiology.* Plenum Press, New York.

JEFFREY, S. W., AND G. F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1, and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* **167:** 191–194.

JOKIEL, P. 1980. Solar ultraviolet radiation and coral reef epifauna. *Science* **207:** 1069–1071.

KAWAGUTI, S. 1941. On the physiology of reef corals V. Tropisms of coral planulae considered as a factor of distribution. *Palao Trop. Biol. Sta. Stud.* **2:** 319–328.

KOTT, P. 1980. Algal-bearing didemnid ascidians in the Indo-West Pacific. *Mem. Queensl. Mus.* **20**(1): 1–47.

KEOUGH, M. J., AND B. J. DOWNES 1982. Recruitment of marine invertebrates: the role of active larval choices and early mortality. *Oecologia* **54:** 348–352.

LAFARGUE, F., AND G. DUCLAUX. 1979. Premier example en Atlantique tropical d'une association symbiotique entre une ascidie didemnidae et une cyanophycee chroococale: *Trididemnum cyanophorum* nov. sp. et *Synechocystis trididemni* nov. sp. *Ann. Inst. Oceanogr. Paris* **55:** 163–184.

LAMBERT, G. 1979. Early post-metamorphic growth, budding and spicule formation in the compound ascidian *Cystodytes lobatus. Biol. Bull.* **157:** 464–477.

LANG, J. C. 1973. Interspecific aggression by scleractinian corals. 2. Why the race is not only to the swift. *Bull. Mar. Sci.* **23:** 260–279.

LEWIN, R. A. 1976. Prochlorophyta as a proposed new Division of algae. *Nature* **261:** 697–698.

LEWIN, R. A., AND N. W. WITHERS. 1975. Extraordinary pigment composition of a prokaryotic alga. *Nature* **256:** 735–737.

LEWIS, J. B. 1974. The settlement behavior of planulae larvae of the hermatypic coral *Favia fragum* (Esper). *J. Exp. Mar. Biol. Ecol.* **15:** 165–172.

LOYA, Y. 1976. Settlement, mortality and recruitment of a Red Sea scleractinian coral population. Pp. 89–100 in *Coelenterate Ecology and Behavior,* G. O. Mackie, ed. Plenum Press, New York.

MAGUIRE, L., AND J. W. PORTER. 1977. A spatial model of growth and competition strategies in coral communities. *Ecol. Model.* **3:** 249–271.

MAST, S. O. 1921. Reactions to light in the larvae of the ascidians, *Amaroucium constellatum* and *Amaroucium pellucidum* with special reference to photic orientation. *J. Exp. Zool.* **34:** 149–187.

MEADOWS, P. S., AND J. I. CAMPBELL. 1972. Habitat selection by aquatic invertebrates. *Adv. Mar. Biol.* **10:** 271–382.

MILLAR, R. H. 1971. The biology of ascidians. *Adv. Mar. Biol.* **9:** 1–100.

MORGAN, T. O. 1977. Growth rate, age at sexual maturity, longevity, and seasonality in three West Indian colonial ascidians. Masters thesis, Univ. Puerto Rico.

MORIARTY, D. J. W. 1979. Muramic acid in the cell walls of *Prochloron. Arch. Microbiol.* **120:** 191–193.

NEWCOMB, E. H., AND T. D. PUGH. 1975. Blue-green algae associated with ascidians of the Great Barrier Reef. *Nature* **253:** 533–534.

OLSON, R. R. 1980. Sun-shade adaptations of a colonial ascidian with a procaryotic symbiont. *Am. Zool.* **20:** 778.

PALMER, A. R., AND R. R. STRATHMANN. 1981. Scale of dispersal in varying environments and its implications for life histories of marine invertebrates. *Oecologia* **48:** 308–318.

RYLAND, J. S. 1974. Behavior, settlement, and metamorphosis of bryozoan larvae. *Thalassia Jugosl.* **10:** 239–262.

SEBENS, K. P. 1981. Recruitment in a sea anemone population: Juvenile substrate becomes adult prey. *Science* **213:** 785–787.

SEEWALDT, E., AND E. STACKEBRANDT. 1982. Partial sequence of 16S ribosomal RNA and the phylogeny of *Prochloron. Nature* **295: 618–620.**

SCOTT, S. F. M. 1954. Metamorphic differentiation in *Amaroucium constellatum* treated with nitrogen mustard. *J. Exp. Zool.* **127:** 331–365.

SHEPPARD, C. R. C. 1979. Interspecific aggression between reef corals with reference to their distribution. *Mar. Ecol. Prog. Ser.* **1:** 237–247.

SOKAL, R., AND F. ROHLF. 1969. *Biometry.* W. H. Freeman Co., San Francisco, CA. 776 pp.

VAN NAME, W. G. 1945. The North and South American ascidians. *Bull. Am. Mus. Nat. Hist.* **84:** 1–45.

WATANABE, H., AND C. C. LAMBERT. 1973. Larva release in response to light by the compound ascidians *Distaplia occidentalis* and *Metandrocarpa taylori. Biol. Bull.* **144:** 556–566.

WHATLEY, J. M. 1977. The fine structures of *Prochloron. New Phytol.* **79:** 309–313.

YAMAGUCHI, M. 1975. Growth and reproduction cycles of the marine fouling ascidians *Ciona intestinalis, Styela plicata, Botrylloides violaceus,* and *Leptoclinum mitsukurii* at Aburatsubo-Moroiso Inlet (Central Japan). *Mar. Biol.* **29:** 253–259.

ZAR, J. H. 1974. *Biostatistical Analysis.* Prentice-Hall, Inc. Englewood Cliffs, NJ. 620 pp.